Pacific Science (1993), vol. 47, no. 3: 256–262

Behavioral Basis of Depth Regulation in the First Zoeal Stage of the Pacific Shore Crab, *Hemigrapsus oregonensis* (Brachyura: Grapsidae)[1]

MARIELISE ARANA[2,3] AND STEPHEN SULKIN[2,4]

ABSTRACT: The behavioral basis of depth regulation is determined for the first pelagic larval stage of the shore crab *Hemigrapsus oregonensis* Dana. Larvae are negatively buoyant, passively sinking at 0.79 cm/sec in 25 parts per thousand (ppt) salinity (S) seawater and at 0.67 cm/sec in 30 ppt S. At 30 ppt S, larvae are negatively geotactic and move upward. At 25 ppt S, larvae remain negatively geotactic, but a low level of locomotor activity results in net downward movement. Swimming speed is higher at 30 ppt S than at 25 ppt S; however, there is no response to incremental increases in hydrostatic pressure up to 0.8 atm at either salinity. Behavioral responses should promote upward migration of the hatching stage similar to the case with other intertidal crab species; however, low precision in depth regulation contrasts with results from other species.

IN MANY BENTHIC marine invertebrates, including most brachyuran crabs, adult distribution is influenced by dispersal of the pelagic larval stage. The pattern of larval dispersal is regulated by a number of factors, including larval morphology, duration, and behavior. In estuarine and coastal marine environments, current speed and direction often vary with depth. As a consequence, patterns of vertical distribution can significantly influence larval dispersal (e.g., Bousfield 1955, Thorson 1964, Cronin 1982). It is well documented that larval crabs possess behavioral traits that contribute to depth regulation (reviewed by Sulkin 1984), thus producing characteristic patterns of vertical distribution and dispersal.

Particular attention has been paid to the behavioral response of the first zoeal stage of many species of crabs (Sulkin 1984). Such studies have documented the presence of behavioral traits that promote upward movement into the water column, a response that presumably results in dispersal of offspring from the site of hatching. Hatching-stage larvae generally respond to the higher pressures and salinities present at depth by increasing locomotor activity. This locomotor activity is oriented by negative geotaxis such that upward migration results. Key behavioral elements of this response thus include negative geotaxis and high barokinesis (Sulkin et al. 1980, Jacoby 1982, Kelly et al. 1982, Schembri 1982). The effects of other environmental parameters, such as salinity, on larval behavior also have been documented (Latz and Forward 1977, Sulkin et al. 1980).

With the exception of Jacoby's (1982) study of larval behavior in *Cancer magister* Dana, most research has been conducted either on shallow-water, estuarine species (Sulkin 1984) or on oceanic species whose adults occupy deep water (Kelly et al. 1982). Both groups possess larval adaptations likely to promote upward migration upon hatching, although some differences in larval behavior between shallow-water and oceanic species have been noted.

The study reported here examined the behavioral responses to gravity and hydrostatic pressure in the hatching stage of the crab *Hemigrapsus oregonensis* Dana, a common shore crab that inhabits rocky intertidal

[1] The first author was supported on this project by a grant to the Shannon Point Marine Center from the National Science Foundation as part of the Research Experiences for Undergraduates Site Program (OCE-9000676). Manuscript accepted 8 October 1992.

[2] Shannon Point Marine Center, 1900 Shannon Point Road, Anacortes, Washington 98221.

[3] 86-24 144th Street, Briarwood, New York 11435.

[4] Correspondent.

beaches along the eastern Pacific Ocean. In the Puget Sound basin, adults occupy the shallow waters of the lower intertidal, but larvae are readily dispersed to nearby waters exceeding 100 m in depth. Results are compared with those reported in the literature for shallow-water species whose larvae are retained in shallow water and for oceanic species whose larvae are distributed in waters of substantial depth.

MATERIALS AND METHODS

Experimental Animals

Ovigerous *H. oregonensis* were collected from the intertidal zone at the Shannon Point Marine Center near Anacortes, Washington. Adult crabs were held in large glass bowls containing filtered seawater at 30 parts per thousand (ppt) salinity (S) and 15°C, until larvae hatched. Larvae from three broods were used in the experiment, with larvae from each brood maintained separately. Upon hatching, larvae from each brood were transferred by pipette to bowls containing clean seawater. Each culture bowl contained 100 larvae. Larvae were transferred daily to clean seawater (30 ppt S; 15°C) and fed newly hatched nauplii of brine shrimp, *Artemia* sp.

Larvae to be used in the various behavioral experiments were drawn randomly from the mass cultures. Larvae used in all experiments that called for 25 ppt S seawater were placed in this lower salinity for at least 12 hr before the experiment. All experiments were conducted on larvae within 24 hr of hatching.

Buoyancy

Buoyancy was measured on anesthetized larvae that had been exposed to seawater of the appropriate salinity to which a few crystals of magnesium sulfate had been added. The larvae were then pipetted gently into a vertical cylinder filled with seawater of either 25 ppt or 30 ppt S as appropriate. Descent rates for 50 larvae were measured in both 25 ppt and 30 ppt S by determining the time required for individuals to traverse a 10-cm-long distance marked off in the middle of the cylinder. Mean values were compared using a Student's *t* test.

Geotaxis

Geotaxis, or gravity orientation, is defined as oriented movement in the vertical plane in the absence of other environmental cues (Fraenkel and Gunn 1961). Two methods were employed to measure geotaxis in *H. oregonensis*.

The first method involved recording the response of individual larvae. A larva was pipetted into a test tube (150 mm long by 15 mm diam.) filled with seawater of either 25 ppt S or 30 ppt S. The test tube was sealed and placed in the horizontal position on a ringstand, with a rotating clamp. The larva was retained in this position for 75 sec in the dark (Forward 1985). The tube was then rotated 90° to the vertical position and the individual was monitored for movement in the vertical plane for the first 10 sec. If a larva moved upward ($\pm 22.5°$ from the vertical axis), it was defined as exhibiting negative geotaxis; if it moved downward ($\pm 22.5°$ from the vertical axis), it was defined as showing positive geotaxis. At each salinity, a total of 25 individual measurements were made for each of three broods ($n = 75$). The numbers responding as negative, positive, or neutral are presented as percentages.

A second method of testing for geotaxis involved placing a sample of 10 larvae into a vertically oriented acrylic observation chamber (30 cm long by 5 cm diam.), divided vertically into three sections of equal length. The larvae were introduced carefully by pipette into the middle section after dark-adaptation for 75 sec. Distribution of larvae among the three sections was determined every 5 min for 30 min. A deep red backlight was used to silhouette the larvae for counting. The experiment was performed with larvae from each of the three broods at each salinity, and the data were pooled for analysis.

Barokinesis

Swimming speed as a function of hydrostatic pressure was determined according to

the method of Kelly et al. (1982). Approximately 25 larvae were placed in the acrylic observation chamber, and the chamber was filled with seawater, sealed, and attached to a closed system in which pressure can be increased. The pressure generator consists of a piston screw pump that develops pressure within a small volume by fluid compression. In these experiments, larvae were subjected to stepwise increments of 0.2 atmospheres (atm), up to a total of 0.8 atm. Larvae were induced to swim along the axis of the horizontally oriented chamber toward a dim white light. Individuals were timed as they swam across a 5-cm space marked off in the middle of the chamber. After 10 measurements were made, the pressure was increased by 0.2 atm to the next increment, and 10 larvae again were timed. The complete series of measurements was repeated three times at each salinity. For each salinity, the mean swimming speed at each pressure was calculated and compared among pressures.

RESULTS

Buoyancy

Anesthetized larvae were negatively buoyant, sinking when placed in either 25 ppt S or 30 ppt S. Mean rate of passive sinking was 0.79 cm/sec in 25 ppt S and 0.67 cm/sec in 30 ppt S. A Student's t test indicated significant differences between the mean values ($P < 0.05$).

Geotaxis

Newly hatched *H. oregonensis* larvae showed clear evidence of negative geotaxis at 30 ppt S. Table 1 shows the results of experiments in which responses of individual larvae were measured. In two of the three experiments, virtually all of the larvae moved upward and none moved downward. In the third experiment, upward movement was shown by 44%, while only 12% moved downward. Figure 1 shows the results of the experiment in which larvae were placed in the middle of a vertical cylinder and their subsequent distribution determined at 5-min intervals. Shown are the results at 5, 15, and 30 min. After only 5 min, a stable distribution was obtained, with the majority moving to the top section of the cylinder.

TABLE 1

PERCENTAGE OF INDIVIDUAL LARVAE MOVING UP, DOWN, OR NOT MOVING IN EITHER DIRECTION (NEUTRAL) IN THREE EXPERIMENTS USING LARVAE FROM EACH OF THREE BROODS, CONDUCTED AT 30 PPT SALINITY AND AT 25 PPT SALINITY

	30 ppt			25 ppt		
DIRECTION OF MOVEMENT	EXPERIMENT NO. 1	2	3	EXPERIMENT NO. 1	2	3
Up	88	96	44	0	32	36
Neutral	12	4	44	8	12	4
Down	0	0	12	92	56	60

Results at 25 ppt S were more equivocal. As shown in Table 1, initial movement of a majority of individuals was downward, although in two of the experiments, approximately one-third of the larvae did move upward. In the experiments using the vertical cylinder, all larvae had positioned themselves in the bottom third of the chamber after 5 min and remained there (Figure 1). The results indicate that negative geotaxis was present in at least some larvae at 25 ppt S. The predominant downward response, however, could be attributed either to positive geotaxis, representing active oriented response, or to a passive response resulting from sinking due to negative buoyancy.

If downward movement at 25 ppt S was due to positive geotaxis, descent rates of unanesthetized larvae should have exceeded the passive sinking rates measured for those that had been anesthetized. Measurements indicated that mean descent rates of unanesthetized larvae were only 0.22 cm/sec ($n = 10$), significantly *lower than* the mean passive sinking rate of 0.79 cm/sec (Student's t test, $P < 0.05$). Direct observation of the larvae indicated that descent was often interrupted by swimming activity that either was nondirected or resulted in upward movement, thus lowering the net rate of descent. Thus, the downward

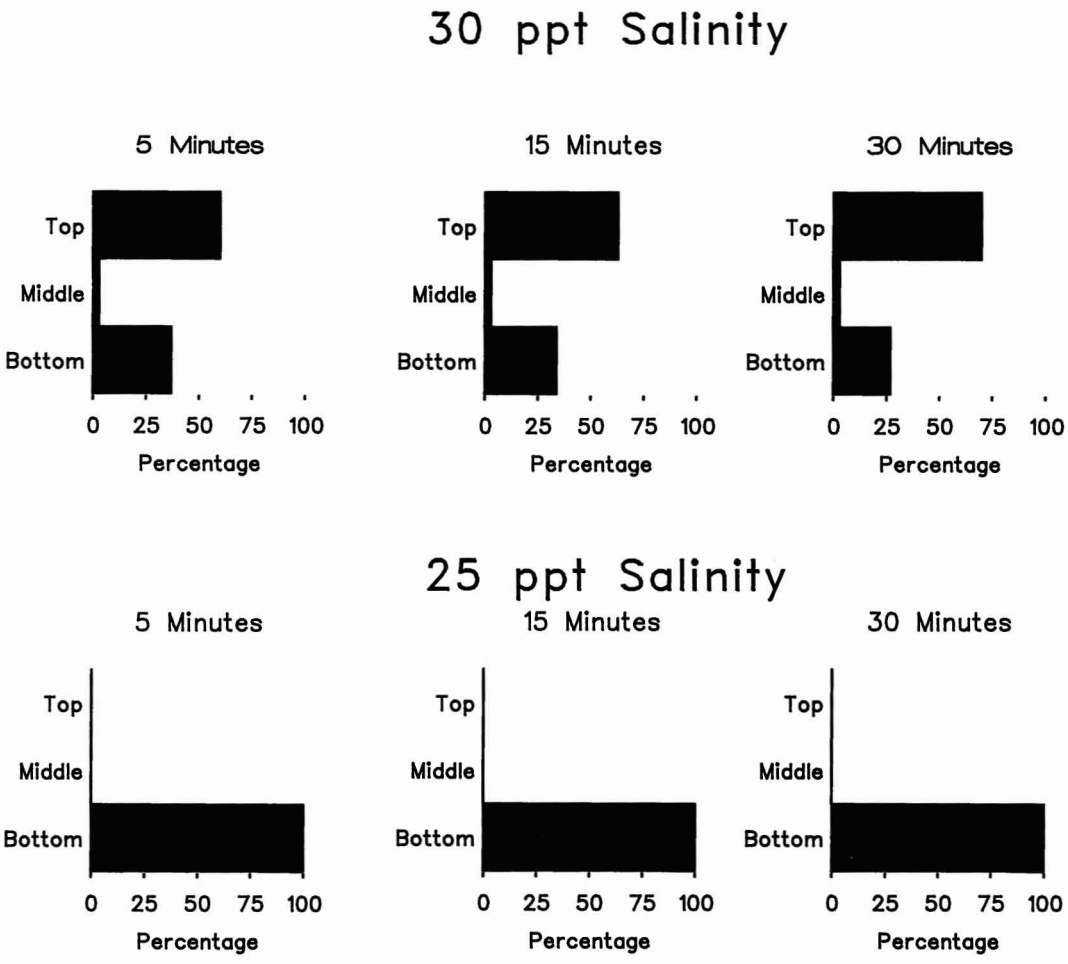

FIGURE 1. Results of geotaxis experiments with *Hemigrapsus oregonensis* conducted at 30 ppt and 25 ppt salinity. Values are presented at 5-, 15-, and 30-min intervals. Kite diagrams represent proportional distributions of 30 larvae among three sections of a vertically oriented chamber.

movement of larvae at 25 ppt S cannot be attributed to positive geotaxis.

Barokinesis

Table 2 shows mean swimming speeds of larvae in response to stepwise increments of pressure at 25 ppt S and 30 ppt S. For larvae tested at each salinity, there was no significant difference in swimming speeds among the pressures tested (25 ppt: $P > 0.12$; 30 ppt: $P > 0.57$). Swimming speed at 30 ppt S exceeded that at 25 ppt S. A Student's t test

TABLE 2

MEAN SWIMMING SPEEDS (CM/SEC) FOR LARVAE AT EACH OF THE INDICATED PRESSURE INCREMENTS TESTED AT 25 PPT AND 30 PPT SALINITY

PRESSURE TESTED	SALINITY	
(atm)	25 ppt	30 ppt
Ambient	0.71	1.78
0.2	0.57	1.86
0.4	0.43	1.50
0.6	0.55	1.42
0.8	0.42	1.54

comparing pooled values for all pressures at each salinity showed significant differences in swimming speed between the two salinites ($P < 0.001$).

DISCUSSION

The hatching stage of *H. oregonensis* shows both similarities and differences in behavioral traits when compared with other intertidal species tested (Sulkin 1984). Stage I *H. oregonensis* zoeae are negatively buoyant and negatively geotactic. These are common traits that form the basis for depth regulation in other brachyuran species, such as *Eurypanopeus depressus* (Smith) (Sulkin et al. 1983) and *Rhithropanopeus harrisii* (Gould) (Forward and Wellins 1989). Oriented by a negative response to gravity, locomotor activity should result in upward movement if its frequency and speed overcome the effects of negative buoyancy. This is the fundamental response that accounts for movement to the surface by the hatching stage of many brachyuran crab species (Sulkin 1984).

A key element in depth regulation, thus, is level of locomotor activity. Upward movement and/or maintenance of position in the water column by negatively buoyant animals requires a level of properly oriented locomotor activity that overcomes sinking. Level of locomotor activity in brachyuran larvae may be influenced by a number of stimuli (reviewed by Sulkin 1984). Among stimuli frequently studied are salinity, a depth-related variable in many estuarine and coastal marine systems (Sulkin et al. 1980), and hydrostatic pressure, a stimulus that varies with depth in a ubiquitous and predictable manner (Hardy and Bainbridge 1951, Bentley and Sulkin 1977, Wheeler and Epifanio 1978, Sulkin et al. 1980, 1983, Jacoby 1982, Kelly et al. 1982, Forward and Wellins 1989).

In the study reported here, salinity influenced the ability of *H. oregonensis* larvae to regulate their depth in an experimental vertical chamber. Results indicated that negative geotaxis was exhibited by larvae at both 25 ppt S and 30 ppt S. However, at 25 ppt S, the majority of larvae moved downward, a response attributed to a comparatively low level of locomotor activity that could not overcome the tendency to sink passively. The result was a net downward shift in position (Figure 1). By contrast, at 30 ppt S, there was a net upward movement (Figure 1), reflecting a level of oriented locomotor activity that was sufficient to overcome negative buoyancy in a majority of larvae. This result is consistent with that determined for stage I zoeae of the blue crab, *Callinectes sapidus* Rathbun, in which increased swimming speed was measured in 35 ppt S as compared with 25 ppt S (Sulkin et al. 1980).

The study reported here demonstrates that stage I *H. oregonensis* larvae differ from larvae of many other shallow-water brachyuran species with respect to their response to small changes in hydrostatic pressure. In contrast to results from these other species (Rice 1964, 1966, Bentley and Sulkin 1977, Wheeler and Epifanio 1978, Sulkin et al. 1983, Sulkin 1984, Forward and Wellins 1989), there is no evidence in *H. oregonensis* of increased swimming speed with a pressure increase up to 0.8 atm above ambient. These results, however, are similar to those reported by Sulkin et al. (1980) for *Callinectes sapidus* and by Kelly et al. (1982) for the deep-sea red crab, *Geryon quinquedens* Smith. In the former case, larvae did not respond to pressures less than 1.0 atm, but did show high barokinesis in higher pressures. In the latter case, no pressure response was seen in pressures up to 20 atm above ambient at 15°C, although a modest increase in swimming speed was measured at 11°C at 10 atm.

Stage I larvae of the estuarine crab *Callinectes sapidus* typically are found in the shelf waters off the east coast of North America (Smyth 1980, Provenzano et al. 1983). Indeed, this species possesses a combination of traits that assures export of larvae from the estuarine hatching zone to the open ocean where zoeal development occurs (Sulkin et al. 1980, Provenzano et al. 1983, Epifanio et al. 1984). *Geryon quinquedens* larvae hatch at the shelf-slope break at depths of 200–400 m (Haefner 1978) and remain in shelf or slope waters.

Among species tested to date (Sulkin 1984, Forward and Wellins 1989), sensitivity to

small changes in hydrostatic pressure is common among species whose larvae may be found in both comparatively shallow estuarine systems and the deeper waters of the open ocean. The absence of such sensitivity to small changes in hydrostatic pressure has been reported for three species, including one oceanic species (*Geryon quinquedens*), one estuarine species (*Callinectes sapidus*), and *H. oregonensis*, an intertidal species. Although these three species are not closely related systematically and occupy very different habitats as adults, larvae of all three species typically occupy waters of considerable depth.

Sulkin (1984) proposed a model for depth regulation in negatively buoyant crab larvae in which sensitivity to pressure is important in determining precision of depth regulation. In this negative feedback model, passive sinking subjects the larvae to increasing hydrostatic pressure that stimulates swimming. Negative geotaxis directs the swimming upward, which reduces the hydrostatic pressure and thus larval locomotor activity. Under these circumstances, threshold of barokinesis will determine the vertical range over which such excursions will occur. Forward and Wellins (1989) have confirmed the model experimentally in the crab *Rhithropanopeus harrisii*.

It is tempting to speculate that high precision in depth regulation may not be universally important among species whose stage I larvae are distributed in waters of considerable depth, as compared with species whose larvae occupy more shallow areas where vertical stratification may occur over shorter ranges. First-stage larvae of the three species in which sensitivity to small changes in hydrostatic pressure is absent do live in deep waters. However, some oceanic species also show high barokinesis at low pressure thresholds. Furthermore, larvae of *Callinectes sapidus* are reported to remain at or very near the surface, indicating a high precision in their ability to regulate depth in spite of a relatively high barokinesis threshold (Smyth 1980, Provenzano et al. 1983). It is apparent that traits other than barokinesis may promote a high level of locomotor activity and can compensate for lack of pressure sensitivity.

Stage I *H. oregonensis* larvae are negatively geotactic and thus will move to the surface, given a sufficient level of locomotor activity. Although our results indicate that pressure response is unlikely to be effective in stimulating such activity, the base level of locomotor activity present at 30 ppt, a salinity typical where larvae are found, will produce upward movement and maintenance of position high in the water column, a phenomenon characteristic of many stage I brachyuran larvae (Sulkin 1984).

ACKNOWLEDGMENT

We thank Gene McKeen for his technical assistance.

LITERATURE CITED

BENTLEY, E., and S. D. SULKIN. 1977. The ontogeny of barokinesis during the zoeal development of the xanthid crab *Rhithropanopeus harrisii* (Gould). Mar. Behav. Physiol. 4:275–282.

BOUSFIELD, E. L. 1955. Ecological control of the occurrence of barnacles in the Miramichi estuary. Natl. Mus. Can. Bull. 137:1–69.

CRONIN, T. W. 1982. Estuarine retention of larvae of the crab *Rhithropanopeus harrisii*. Estuarine Coastal Shelf Sci. 15:207–220.

EPIFANIO, C. E., C. C. VALENTI, and A. E. PEMBROKE. 1984. Dispersal and recruitment of blue crab larvae in Delaware Bay, U.S.A. Estuarine Coastal Shelf Sci. 18:1–12.

FORWARD, R. B., JR. 1985. Behavioral response of larvae of the crab *Rhithropanopeus harrisii* (Brachyura: Xanthidae) during diel vertical migration. Mar. Biol. (Berl.) 90:9–18.

FORWARD, R. B., JR., and C. A. Wellins. 1989. Behavioral responses of a larval crustacean to hydrostatic pressure: *Rhithropanopeus harrisii* (Brachyura: Xanthidae). Mar. Biol. (Berl.) 101:159–172.

FRAENKEL, G. S., and D. L. GUNN. 1961. The orientation of animals. Dover Publ., New York.

HAEFNER, P. A., JR. 1978. Reproductive biology of the female deep sea red crab *Geryon quinquedens*, from the Chesapeake Bight. U.S. Fish Wildl. Serv. Fish. Bull. 75:91–102.

HARDY, A. C., and R. BAINBRIDGE. 1951. Effect of pressure on the behavior of decapod larvae. Nature (Lond.) 167:354–355.

JACOBY, C. A. 1982. Behavioral responses of the larvae of *Cancer magister* Dana (1852) to light, pressure, and gravity. Mar. Behav. Physiol. 8:267–283.

KELLY, P., S. D. SULKIN, and W. F. VAN HEUKELEM. 1982. A dispersal model for larvae of the deep sea red crab *Geryon quinquedens* Smith based upon behavioral regulation of vertical migration in the hatching stages. Mar. Biol. (Berl.) 72:35–43.

LATZ, M. I., and R. B. FORWARD, JR. 1977. The effect of salinity upon phototaxis and geotaxis in a larval crustacean. Biol. Bull. (Woods Hole) 153:163–179.

PROVENZANO, A. J., JR., J. R. MCCONAUGHA, K. B. PHILLIPS, D. F. JOHNSON, and J. CLARK. 1983. Vertical distribution of the first stage larvae of the blue crab, *Callinectes sapidus*, at the mouth of Chesapeake Bay. Estuarine Coastal Shelf Sci. 16:489–499.

RICE, A. L. 1964. Observations on the effects of changes in hydrostatic pressure on the behavior of some marine animals. J. Mar. Biol. Assoc. U.K. 44:163–175.

———. 1966. The orientation of pressure responses of some marine Crustacea. Pages 1124–1131 *in* Proceedings of the Symposium on Crustacea, Part III. Marine Biology Association of India.

SCHEMBRI, P. J. 1982. Locomotion, feeding, grooming, and the behavioral responses to gravity, light, and hydrostatic pressure in the Stage I larvae of *Ebalia tuberosa* (Crustacea: Decapoda: Lencosiidae). Mar. Biol. (Berl.) 72:125–134.

SMYTH, P. O. 1980. *Callinectes* (Decapoda: Portundae) larvae in the middle Atlantic Bight, 1975–77. U.S. Fish. Wildl. Serv. Fish. Bull. 78:241–265.

SULKIN, S. D. 1984. Behavioral basis of depth regulation in the larvae of brachyuran crabs. Mar. Ecol. Prog. Ser. 15:181–205.

SULKIN, S. D., W. F. VAN HEUKELEM, and P. KELLY. 1983. Behavioral basis of depth regulation in the hatching and post-larval stages of the mud crab *Eurypanopeus depressus* Hay and Shore. Mar. Ecol. Prog. Ser. 11:157–164.

SULKIN, S. D., W. F. VAN HEUKELEM, P. KELLY, and L. VAN HEUKELEM. 1980. The behavioral basis of larval recruitment in the crab *Callinectes sapidus* Rathbun, a laboratory investigation of ontogenetic changes in geotaxis and barokinesis. Biol. Bull. (Woods Hole) 159:402–417.

THORSON, G. 1964. Light as an ecological factor in the dispersal and settlement of larvae of marine bottom invertebrates. Ophelia 1:167–208.

WHEELER, D., and C. E. EPIFANIO. 1978. Behavioral response to hydrostatic pressure in larvae of two species of xanthid crabs. Mar. Biol. (Berl.) 46:167–174.